IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Yoshihisa NAITO

Confirmation: 1812

Serial No.: 10/553,256

Group Art Unit: 1612

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Examiner: Sabiha Naim QAZI

For: METHOD OF PREVENTING, CURING, AND/OR TREATING HYPOCALCEMIA OF A DOMESTIC MAMMAL

DECLARATION SUBMITTED UNDER 37 C.F.R. 1.132

Commissioner for Patents
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1. I, Norio Yamagishi, make the following statements based on personal knowledge;

2. My address is Chat Wood-B, Higashi-Aniwa 13-53, Morioka, Iwate , JAPAN 020-0824.

3. I am familiar with the above-identified application, including the currently pending claims thereof, and have no financial interest in the above-referenced patent application or any resulting patent issued from allowance of this application;

4. My educational background is as follows:

a). I received my Bachelor of Veterinary Medicine (Doctor of Veterinary Medicine) from Rakuno Gakuen University, Japan (faculty of Veterinary Medicine) in March of 1993;

b). I received my Ph.D from Gifu University, Japan (United Graduate School, Division of Veterinary Medicine) in March 1999.

5. My research history is as follows:

a). April 1993 · May 1996 Graduate student

Division of Veterinary Medicine, United Graduate School · Gifu University, Japan

b). July 1996 · January 2003 Assistant Professor

Department of Veterinary Medicine, Obihiro University of Agriculture & Veterinary Medicine, Japan

c). February 2003 · May 2005 · Associate Professor

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d). June 2005 · January 2008 · Associate Professor

Department of Clinical Veterinary Medicine

Faculty of Agriculture, Iwate University, Japan

e). February 2008 · Present · Professor

Department of clinical Veterinary Medicine

Faculty of Agriculture, Iwate University, Japan

6. In order to demonstrate dose-dependent absorption of 1,25-dihydroxyvitamin D_3 and the bioavailability of the same when administered to a cow, the following experiments where conducted under my supervision and control.

(A) DOSE DEPENDENCE

1. The present Invention

Reaction tests based on doses of 1,25-dihydroxyvitamin D_3 (hereinafter · 1,25-$(OH)_2D_3$ · or · calcitriol ·) were carried out on five holstein cows (age:3-9 years, body weight: 616 to 804 kg) by transvaginal administration.

The transvaginal administration level of 1,25-$(OH)_2D_3$ for the five cows are 0.125, 0.25, 0.5, and 1.0 · g/kg (body weight ratio), respectively. The intravenous injection of 1,25-$(OH)_2D_3$ was conducted at a level of 1.0 · g/kg (bodyweight ratio) at longer-than-fortnight intervals by using 5x5 Latin square method.

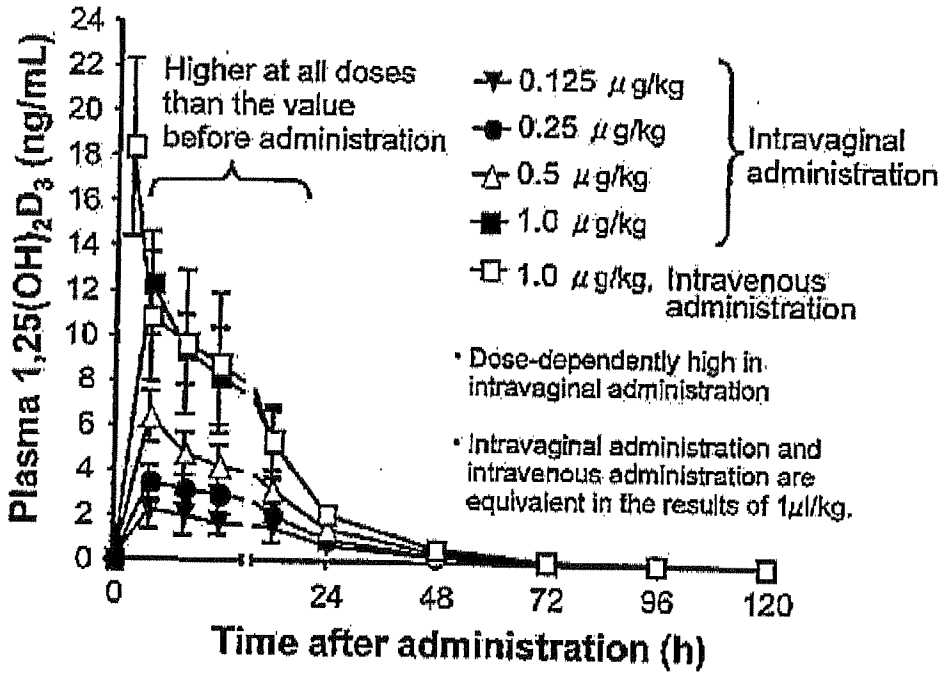
The 1,25-$(OH)_2D_3$ (product of Mercian Corporation) used in the tests, having a form of crystalline powder, was dissolved in 99% ethanol to be a concentration of 200μg/mL and then frozen for preservation at -20 ·C until used.

A drug composed of 5 mL of 20% ethanol solution containing 1,25-$(OH)_2D_3$ at 0.125, 0.25, 0.5, or 1.0 · g/kg (by body weight) was administered to a vaginal lumen using a Split Universal Sheath (IMV Int. CO., France) by a rectovaginal cavity method. The vulva was then bonded with an adhesive in order to prevent the 1,25-$(OH)_2D_3$ solution from being unintentionally excreted from the vaginal lumen. Intravenous administration was performed using a cannula (14-ga cannula for animals, manufactured by Nipro Medical Industries Ltd.) mounted in advance for the collection of a blood sample.

Heparinized blood samples were collected immediately before administration of 1,25-$(OH)_2D_3$ (Hour 0) and Hour 2, 4, 6, 12, 24, 48, 72, 96 and 120 after administration of 1,25-$(OH)_2D_3$.

Next, each of the blood samples was immediately centrifuged, and the concentration of 1,25-$(OH)_2D_3$ in plasma was measured.

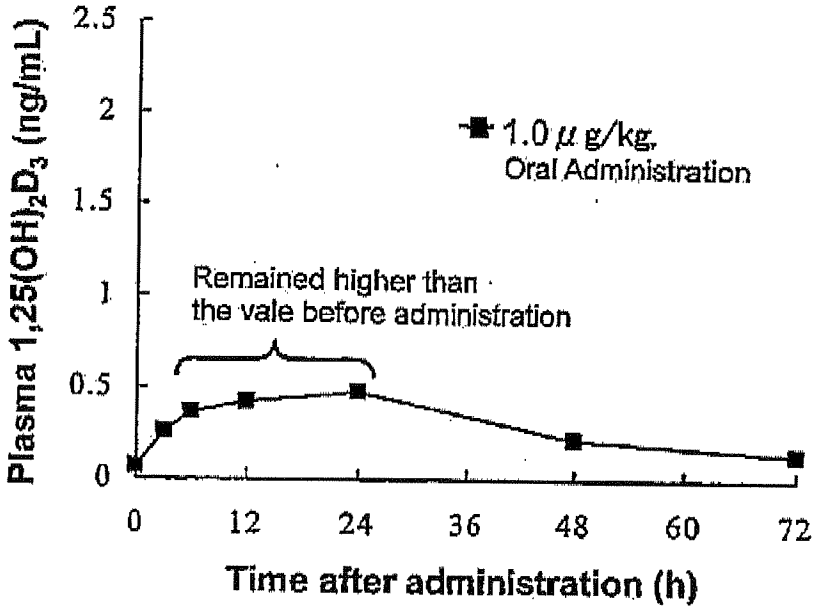
Changes over time (hour-to-hour basis) in the concentration (ng/mL) of 1,25-$(OH)_2D_3$ in blood plasma after transvaginally administrating 1,25-$(OH)_2D_3$ to the cows are shown below (the present invention).



2. Comparative Tests

For comparison, changes over time (hour-to-hour basis) in the concentration (ng/mL) of 1,25-$(OH)_2D_3$ in blood plasma after orally administrating 1,25-$(OH)_2D_3$ to the cows are shown below (Comparative Test). This Comparative Test was carried out on five holstein cows (age: 15-36 months, body weight: 190 to 600 kg) to which 1,25-$(OH)_2D_3$ was orally administered. The five cows were administered with 1.0 · g/kg (body weight ratio) of 1,25-$(OH)_2D_3$ in the same manner with the above tests of the present invention.

Similarly with the above, a drug composed of 10% ethanol solution containing 1,25-$(OH)_2D_3$ at 50 μg/kg (by body weight) was orally administered, and the heparin blood samples were collected to measure the concentration of 1,25-$(OH)_2D_3$ in plasma.



7). The results of the above experiments on the present invention (intravaginal administration) and Comparative tests (oral administration) are as follows:

1) Intravaginal administration of calcitriol

In intravaginal administration of to cows, the concentration of $1,25\text{-}(OH)_2D_3$ in blood reached its peak 2 hours after administration and then declined. In the intravaginal administration, the highest values of blood $1,25\text{-}(OH)_2D_3$ level were 12ng/mL in case of 1.0 • g/kg (calcitriol dose) and 2ng/mL in case of 0.125μg/mL, respectively. These values are 24 times higher and 4 times higher respectively, than the highest value, 0.5ng/mL, of the blood $1,25\text{-}(OH)_2D_3$ level in case of oral administration of calcitriol (1.0 • g/kg) to cows.

2) Oral administration of calcitriol

In case of oral administration of calcitriol (1.0 • g/kg) to cows, the concentration values of $1,25\text{-}(OH)_2D_3$ in blood were significantly high until 6 to 24 hours after administration, as compared with the concentration in blood before administration. In the oral administration (1.0 • g/kg) of to cows, the concentration of $1,25\text{-}(OH)_2D_3$ in blood reached its peak, 05 ng/mL, 24 hours after administration and then declined. Also, as described in Example 3 of the Specification, it is known that no increase in calcitriol level in blood serum in proportion to increases in dosage is observed in case of oral administration. (Muindi et al (2002) ; Pharmacokinetics of high-dose oral calcitriol: Results from a phase I trial of calcitriol and paclitaxel. Clin. Pharmacol. Ther. 72:648-659.)

8. BIOAVAILABILITY

1) Intravaginal administration of calcitriol

As described in Example 3 of the Specification, the bioavailability of $1,25\text{-}(OH)_2D_3$ in transvaginal administration was approx. 93 %.

2) Oral administration of calcitriol

As described in Example 3 of the Specification, it is known that the bioavailability of $1,25\text{-}(OH)_2D_3$ hours after calcitriol was administered at a dose of 60 ng/kg to a young patient dialyzed for a long period was 62%. (Salusky et al. (1990); Pharmacokinetics of calcitriol in continuous ambulatory and cycling peritoneal dialysis patients. Am. J. Kidney Dis. 16:126-32.)

9. Conclusion

(a) Dose-dependent absorption

As shown in above (2)(a) 1), in intravaginal administration of calcitriol to cows, an unexpected effect of "dose-dependent" absorption was observed while no such effect was obtained in case of oral administration of calcitriol. Moreover, absorption in the intravaginal administration was much higher than that in case of oral administration. That is, the case where $1,25\text{-}(OH)_2D_3$ was transvaginally administered to cows according to the present invention shows that $1,25\text{-}(OH)_2D_3$ was absorbed through the vaginal wall dose-dependently and highly efficiently. Therefore, in this regard, the advantage in intravaginal administration of $1,25\text{-}(OH)_2D_3$ is clear as compared with oral administration. Thus, the blood $1,25\text{-}(OH)_2D_3$ level can be enhanced dose-dependently rapidly and efficiently by using an extremely small amount of calcitriol in intravaginal administration to cows, as compared with oral administration. This means that the blood $1,25\text{-}(OH)_2D_3$ level can be controlled more easily in intravaginal administration than oral administration. Accordingly, in clinical practice, occurrence of toxic accidents can be avoided by using a

suitable formulation developed to obtain a requisite minimum pharmacological effect, which also leads to cost reduction.

(b) Bioavailability

Bioavailability of 1,25-$(OH)_2D_3$ in transvaginal administration were not known before the present application was filed. The bioavailability of 1,25-dihydroxyvitamin D_3 in transvaginal administration of 1,25-dihydroxyvitamin D_3 to cows according to the present invention is approximately 93 %, much higher than 62% in case of oral administration.

10. Further declarant sayeth not.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

Norio Yamagishi

July 9, 2008
Date